Tannic Acid Is an Inhibitor of CXCL12 (SDF-1α)/CXCR4 with Antiangiogenic Activity


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ABSTRACT

Purpose: Increasing evidence suggests that interaction between the chemoattractant CXCL12/stromal cell-derived factor-1α and its receptor CXCR4 plays a pivotal role in the metastasis of various tumors. Our previous studies showed that multi-component Chinese herbal medicines inhibited the effects of CXCL12/CXCR4. As a result of sequential chromatographic fractionation of one herbal medicine ingredient, Lianqiao (fruit of Forsythia suspensa), we observed that tannins were, at least in part, responsible for this activity. The aim of this study was to assess the anti-CXCL12/CXCR4 activity of a commercial tannic acid and evaluate its potential to inhibit tumor cell migration and angiogenesis in vitro.

Experimental Design: The inhibitory effect of tannic acid on CXCL12/CXCR4 was measured by chemotaxis assay, ligand binding assay, and fluorescence-activated cell sorter analysis. The antiangiogenic effect of tannic acid was assessed by in vitro endothelial cell tube formation.

Results: Tannic acid, at nontoxic concentrations, specifically inhibited CXCL12-induced human monocyte migration (IC50 7.5 μg/ml) but did not inhibit CCL2-, CCL3-, CCL5-, formylmethionylleucylphenylalanine (fMLP)-, or CSA-induced migration. The compound markedly blocked CXCL12 binding to THP-1 cells (IC50 0.36 μg/ml). Tannic acid also inhibited CXCL12-induced, but not epidermal growth factor-induced, migration of MDA 231 breast tumor cells. Additionally, 0.5 µg/ml of tannic acid selectively inhibited CXCL12-mediated, but not basic fibroblast growth factor- or endothelial cell growth supplement-mediated, bovine aorta endothelial cell capillary tube formation.

Conclusion: These studies indicate that tannic acid is a novel selective CXCL12/CXCR4 antagonist and consequently may provide a mechanistic basis for the reported antitumor and anti-inflammatory properties of tannic acid.

INTRODUCTION

Chemokines constitute a superfamily of small, chemoattractant, cytokine-like proteins that orchestrate immunological and inflammatory processes such as leukocyte trafficking, adhesion, hematopoiesis, and angiogenesis. Interaction of chemokines with specific G protein-coupled receptors on target cells produces their biological effects (1). SDF-1α (CXCL12) is a C-X-C chemokine that interacts with a specific receptor, CXCR4 (2, 3). It is well known that CXCR4 is a coreceptor used by T cell-tropic (X4) HIV strains to enter the host cell (4). Recently, increasing evidence suggests that CXCL12 and CXCR4 promote the spread of cancer cells. Elegant observations by Muller et al. (5) showed that human breast cancer cells express high levels of CXCR4, whereas CXCL12 mRNA is expressed maximally at sites of breast tumor metastasis. Neutralizing antibodies to CXCR4 markedly reduced metastasis. Other tumor cells, such as ovarian cancer cells (6) and prostate cancer cells (7), have also been shown to use CXCL12/CXCR4 interaction to migrate and spread. Additionally, there are reports describing endothelial cell CXCR4 expression in human pancreatic cancer and glioblastoma (8, 9). Stimulation of endothelial cells with CXCL12 resulted in neovessel formation in vitro (10). This evidence suggests that an antagonist of CXCL12/CXCR4 may be a useful therapeutic agent for treatment of tumors as well as HIV infection and inflammation.

In an effort to find naturally occurring chemokine antagonists, we screened a number of Chinese medicinal herbs. One commonly used injectable, anti-inflammatory Chinese herbal preparation, named SHHL, suppressed the chemokine-induced chemotactic response of human monocytes (11). Subsequent evaluation of the herbs contained in SHHL revealed that both aqueous and organic extracts of Lianqiao, the fruit of Forsythia suspensa (Oleaceae), markedly blocked radiolabeled CXCL12 binding to human monocyctic cell line THP-1 cells and inhibited
CXCL12-induced human monocyte migration. Sequential activity-guided chromatographic fractionation indicated that a polyphenol-like tannic acid was the component in Lianqiao with anti-CXCL12 activity. Experiments shown here verified this hypothesis by using commercially available tannic acid.

Tannins are water-soluble polyphenols that are widely distributed in the plant kingdom, including food grains and fruits (12). The tea plant, for example, is a widely used beverage that contains abundant tannic acid [~40 mg/g (13)]. Epidemiological studies have shown a negative association between tea consumption and the incidence of cancers (14, 15). Recent experiments demonstrated that tannic acid and related green tea polyphenols inhibited the mouse mammary tumor virus promoter (16, 17). In addition to its chemopreventive effects, tannic acid also directly suppresses cancer cell growth. In vitro, tannic acid inhibited the proliferation of various cancer cell lines (18–20) and induced cancer cell apoptosis (21–24). Given in drinking water, tannic acid enhanced the survival rate of mice bearing syngeneic tumors (25).

In this study, we provide evidence that tannic acid selectively inhibits CXCL12/CXCR4, and the resultant antiangiogenic property of tannic acid may contribute to its known anticancer activities.

MATERIALS AND METHODS

Reagents and Cells. Tannic acid and related tea polyphenolic compounds were obtained from Sigma (St. Louis, MO). 125I-labeled CXCL12 and 125I-labeled RANTES were purchased from DuPont NEN (Boston, MA). CellTiter 96® AQueous was obtained from Promega (Madison, WI). Human peripheral blood enriched in mononuclear cells was obtained from normal donors by leukapheresis (Transfusion Medicine Department, Clinical Center, NIH, Bethesda, MD, with an approved human subjects agreement). The blood was centrifuged through Ficoll-Hypaque (Sigma), and peripheral blood mononuclear cells collected at the interface were washed with PBS and centrifuged through isosmotic Percoll (Pharmacia, Uppsala, Sweden) gradient. The enriched monocyte populations were obtained at the very top of the gradient (top fraction). The cells were collected and washed twice with ice-cold PBS. Cells were suspended in RPMI 1640 containing 10% (v/v) FBS (HyClone, Logan, UT), 10 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc., Gaithersburg, MD). BAECs were kindly provided by Dr. Adriana Haimovitz (Memorial Sloan-Kettering Cancer Center, New York, NY) and maintained at 10% CO2 in DMEM containing 10% fetal bovine serum supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), l-glutamine (2 mM), and 1 ng/ml bFGF. The MDA231 human breast carcinoma cell line was obtained from American Type Culture Collection and grown in RPMI 1640 containing 10% FCS, glutamine (2 mM), and penicillin-streptomycin (100 U/ml).

Herbal Extract Fractionation. Lianqiao [dried fruit of Forsythia suspensa (Oleaceae)], Shuanghua [flower bud of Lonicer japonica (Caprifoliaceae)], and Huangjin [root of Scutellaria baicalensis (Labiateae)] were bought from Dahsing Pharmaceutical Co. (Washington, DC). Organic and aqueous extracts of herbal medicines were obtained by National Cancer Institute-Frederick standard methods. Briefly, the air dried plant specimens were finely ground, weighed, transferred to a borosilicate glass percolator, covered with 1:1 solution of dichloromethane/methanol, and steeped overnight. Solvent was drained and replaced with methanol, which was then drained and combined with the previous organic solvent extract. After solvent removal by rotary evaporation, the concentrate was vacuum dried prior to biological evaluation. After removal of methanol from the plant material, high purity water was added to the plant material and allowed to steep overnight. The aqueous extract was then lyophilized.

For further fractionating of the organic extract of Lianqiao, 5 g of the organic extract of Lianqiao were coated on 50 g of Dicalite Speed-Plus, a diatomaceous earth of low activity. The coated dicalite was repeatedly treated with solvent and evaporated to yield a dry, flowable powder that was packed in a short glass column and eluted successively with 500 ml each of hexane, ethyl acetate, acetone, and methanol. These fractions were evaporated to yield 1.5 g of hexane eluate, 2.1 g of ethyl acetate eluate, 0.52 g of acetone eluate, and 0.58 g of methanol eluate. Because the methanol eluate was active, half of the methanol eluate (0.29 g) was permeated through a 2.5 × 42-cm column of Sephadex LH-20 in methanol:water (7:3 v/v). A total of five fractions were obtained by combining tubes based on UV absorbance at 230 nm. The earliest eluting fraction (17 mg) was the most active, with residual activity in the second fraction (44 mg). The remaining three fractions were inactive. HPLC of the first fraction on a 10 × 250-mm-wide-pore diol HPLC column (Waters-YMC) in a gradient of methanol:water yielded two fractions, an unretained peak (13.7 mg) that displayed good inhibitory activity and a complex group of UV-absorbing peaks with minimal inhibitory activity (0.8 mg). Batch elution of the unretained fraction through a bed of 2 g of polyamide resin (Brinkmann MN-6) gave a water eluate (1.7 mg) and a methanol eluate (6.1 mg), which were both inactive in the binding assay. Recovery of mass was only 57% of that applied.

Cytotoxicity Assay. Freshly isolated human monocytes, THP-1 cells or BAEC cells, were plated into 96-microwell plates (Costar, Corning, NY) at 1 × 105 cells/well in the presence of tannic acid at a final concentration of 100 μg/ml. Cell viability was determined by [3H]thymidine incorporation (THP-1 cells and BAEC cells) or trypan blue exclusion (human monocytes), 24 h after coincubation at 37°C in humidified air with 5% CO2.

Chemotaxis. Migration of human monocytes and MDA 231 tumor cells was assessed using a 48-well microchemotaxis chamber technique. Chemokines (PreproTech) and chemotactracts (all chemokines were used at 100 ng/ml, FMLP was 10−8 M, and C5a was 10−9 M) were diluted in chemotaxis medium [RPMI 1640, 1% BSA (Sigma, A4301), 25 mM HEPES, for MDA 231 cell chemotactic assay, 0.1% BSA was used] and placed in the lower wells. A 50-μl cell suspension (105 human monocytes/ml and 1 × 105 MDA 231 cells/ml in chemotaxis medium) was placed in the upper wells (Neuroprobe, Cabin John, MD). Tannic acid was added to the cell suspension before the cells were placed in the chamber. A polycarbonate filter was used to separate the two compartments (Neuroprobe; 5-μm pore size for human monocytes and fibronectin precoated 10-μm pore size membrane for MDA 231...
cells). After incubation at 37°C in humidified air with 5% CO₂ (1.5 h for monocytes and 3 h for MDA 231 cells), the filter was removed, fixed, and stained with Diff-Quik (Harlew, Gibbstown, NJ). The cells on the underside of the membrane were counted at ×200. The data were expressed as the migrating cell number/high power field. The percent inhibition of chemotaxis was also calculated by the following formula:

\[ PI = \left( 1 - \frac{EG}{CG} \right) \times 100, \]

where \( EG \) means migratory cell number in experimental group minus spontaneous migration, and \( CG \) means migratory cell number in response to chemokine- and chemoattractant-positive control group minus spontaneous migration.

**Binding Assay.** The cells were suspended in binding medium (RPMI 1640, 1% BSA, and 25 mM HEPES). Triplicate samples of cells (1 × 10⁶) and herbal extracts or tannic acid or medium alone contained the same concentration of ¹²⁵I-labeled CXCL12/SDF-1α (0.12 nM) or ¹²⁵I-labeled CCL5/RANTES (0.12 nM). Samples were incubated at 37°C for 20 min with constant rotation. After incubation, the cells were centrifuged through a 10% sucrose/PBS cushion, and the cell-associated radioactivity was measured in a gamma counter. The percentage of total binding was calculated to equal 100 × cpm (with tannic acid)/cpm (without tannic acid). All experiments were performed at least three times, and the results of a representative experiment are presented. All data are expressed as mean ± SE.

**Flow Cytometry.** The effects of tannic acid on CXCR4 and CD14 surface expression on human monocytes were monitored by fluorescence-activated cell sorter analysis (courtesy of L. Finch, Science Applications International Corporation-Frederick, Inc., National Cancer Institute-Frederick). The cells were pretreated by incubation with 10 μg/ml of tannic acid for 20 min at 37°C and then stained with control FITC-conjugated isotype matched mouse IgG (PharMingen, 20 μl/sample), FITC-conjugated antihuman CXCR4-purified mAb (R&D; 20 μl/sample) or FITC-conjugated antihuman CD14 mAb (PharMingen; 20 μl/sample). Stained cells were analyzed on EPICS profile (Coulter Corp., Miami, FL).

**Capillary Tube Formation.** BAEC tube formation was performed in a 48-well plate. The wells were precoated with 100 μl of Matrigel (BD Biosciences). Confluent BAECs, maintained in 5% FCS, bFGF-free medium for 60 h, were removed from culture, trypsinized, and resuspended at 5 × 10⁵ cells/ml in endothelial basic growth factors medium (Clonetics, Walkers-
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RESULTS

Extracts of Lianqiao Inhibit CXCL12-induced Chemotaxis and CXCL12 Binding with CXCR4. Because SHHL, a multiple herbal preparation, inhibited certain chemokine/chemoattractant-directed chemotaxis (11), the effects of three ingredient herbs on human monocyte migration were investigated. At a concentration of 250 μg/ml, the aqueous extracts of Lianqiao completely abrogated CXCL12-induced chemotaxis, whereas aqueous extracts of Huangqin and Shuanghua did not have significant inhibitory action (Fig. 1). Both aqueous and organic extracts of Lianqiao markedly suppressed CXCL12-induced human monocyte migration, but they did not influence CCL2-, CCL3-, and C5a-directed human monocyte chemotaxis (Fig. 2, A and B). The inhibitory effect of Lianqiao extracts on CXCL12-mediated chemotaxis was dose dependent (Fig. 2, C and D), and the IC50 for aqueous extract was <31.3 μg/ml, whereas the IC50 for organic extract was ~25 μg/ml. The effects of Lianqiao extracts are not based on its toxicity (data not shown).

The competitive binding assay showed that both aqueous and organic extracts at 10 μg/ml of Lianqiao significantly blocked 125I-labeled CXCL12 binding to THP-1 cells; the percent inhibition was 62 and 70%, respectively (Fig. 3A). At the same concentration, both aqueous and organic extracts did not block 125I-labeled CCL5 binding to THP-1 cells (Fig. 3B). These results suggest that the inhibitory effects of Lianqiao extracts on CXCL12-induced chemotaxis resulted from their blockade of CXCL12 binding to its receptor.

Monitored by the binding assay, we systematically refined the active component in organic extract of Lianqiao. Among eluates of hexane, ethyl acetate, acetone, and methanol, only the methanol eluate was active in the binding assay at 10 μg/ml. A total of five fractions were obtained when the methanol eluate was permeated through a Sephadex LH-20 column in methanol:water (1:1, v/v). The earliest eluting fraction was the most active, with 97% inhibition at 10 μg/ml, with residual activity in the second fraction (27% inhibition at 10 μg/ml). The remaining three fractions were inactive. HPLC of the first fraction yielded two fractions, an unretracted peak that displayed good inhibitory activity (73% inhibition at 10 μg/ml) and a complex group of UV-absorbing peaks with minimal inhibitory activity (19% inhibition at 10 μg/ml). Batch elution of this unretracted fraction through a bed of polyamide resin gave a water eluate and a methanol eluate, which were both inactive in the binding assay. Recovery of mass was only 57% of that applied, indicating the inhibitory activity of the parent fraction was likely to be attributable to polyphenolic tannins that bound irreversibly to the polyamide resin (26).
Cytotoxic Effects of Tannic Acid on the Cells. Coincubation for 24 h with up to 10 μg/ml tannic acid did not decrease THP-1 cell viability. However, 50 μg/ml tannic acid significantly suppressed [3H]thymidine incorporation by THP-1 cells (P < 0.01). Up to 50 μg/ml of tannic acid did not exhibit any lytic effects on human monocytes. Less than 10 μg/ml tannic acid did not influence BAEC cell proliferation, but as concentrations reached 50 μg/ml, tannic acid almost completely inhibited BAEC proliferation (P < 0.001; Fig. 4). Because the cytotoxic assay for nonproliferative cells (monocyte) is different from that for proliferative cells (THP-1 and BAECs) in this study, it is hard to conclude that monocytes are more resistant to tannic acid.

Effects of Tannic Acid on Human Monocyte Chemotactic Response to Chemokines and Other Chemoattractants. Human monocytes express abundant chemokine receptors including CXC chemokine receptors (CXCR1, CXCR2, and CXCR4), CC chemokine receptors (CCR1, CCR2, CCR4, CCR5, and CCR8), CX3CR1, and classic chemoattractant receptors (CSaR, C3aR, and FPRs). Using a micro-Boyden chamber, the effects of tannic acid on human monocyte migration were investigated. Tannic acid selectively inhibited CXCL12-induced migration. A, dose response of tannic acid on CXCL12-induced monocyte migration. Compared with control group (chemokine or chemoattractant only): ***, P < 0.001. Bars, SE.

Fig. 5 Effect of tannic acid on chemokine- and chemoattractant-induced human monocyte chemotaxis. Chemokines and chemoattractants were placed in the lower wells of the chemotaxis chamber. Cell suspension was placed in the upper wells with/without tannic acid. Polycarbonate filters separated the upper and lower wells. After incubation, the cells that migrated across the filters were stained and counted. A, tannic acid selectively inhibited CXCL12-induced migration. B, dose response of tannic acid on CXCL12-induced monocyte migration. Compared with control group (chemokine or chemoattractant only): ***, P < 0.001. Bars, SE.

Fig. 6 Dose-dependent, reversible inhibition by tannic acid of radio-labeled CXCL12 (A and B) binding to THP-1 cells and the percent inhibition of tannic acid and related tea polyphenolic compounds on CXCL12 binding to THP-1 cells (C). THP-1 cells (0.5 × 10^6 cells/200 μl) were incubated with increasing concentrations of tannic acid and a constant concentration of 125I-labeled CXCL12 at 37°C for 20 min with constant mixing. After incubation, the cells were centrifuged through a 10% sucrose/PBS cushion, and the cell-associated radioactivity was measured in a gamma counter. Unlabeled CXCL12 was used as a control competitor (A). In another experiment, cells were pretreated with 10 μg/ml of tannic acid for 30 min at room temperature, followed by a thorough washing with PBS three times. One set of cells recovered volume with medium alone [TA(wash)], whereas another set of cells added 10 μg/ml tannic acid [TA(wash) + TA] (B). The cells treated with 10 μg/ml tannic acid or 10 μM related tea polyphenolic compounds (C). Binding of 125I-labeled CXCL12 to the cells was determined. The percentage of total binding or percent inhibition of total binding was calculated, as described in “Materials and Methods.” Data are expressed as the means (bars, SE; n = 3).
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**Effects of Tannic Acid on Anti-CXCR4 Antibody Binding with Human Monocytes.** The data from the competitive ligand binding assays suggested that tannic acid might interfere with CXCL12 binding to its cellular receptor, but whether tannic acid binds CXCL12 or with its receptors remained to be determined. To clarify this, we assessed the effects of tannic acid on the ability of a CXCR4 mAb to detect CXCR4 on cell surfaces. As shown in Fig. 7, freshly isolated human monocytes treated with 10 μg/ml of tannic acid resulted in a 50% reduction in antibody binding (Fig. 7A). In contrast, treatment with the same concentration of tannic acid did not inhibit, but slightly enhanced, the binding of anti-CD14 to freshly isolated human monocytes (Fig. 7B).

**Effects of Tannic Acid on CXCL12-induced Chemotaxis of MDA231 Breast Tumor Cells.** MDA231 breast tumor cells express functional CXCR4. CXCL12-induced MDA231 chemotaxis and invasive responses have been shown to play an important role in breast tumor metastasis (5). Consistent with Muller’s observation, in our experimental system MDA231 cells migrated toward CXCL12. MDA231 cells also migrate toward EGF. Just as what we observed with human monocytes, 10 μg/ml of tannic acid significantly inhibited CXCL12-induced chemotaxis. Tannic acid did not significantly influence EGF-induced chemotaxis, suggesting that the inhibitory effect of tannic acid is selective and not based on its toxic effects (Fig. 8).

**Effects of Tannic Acid on BAEC Tube Formation Induced by CXCL12, Basic Fibroblast Growth Factor, and ECGS.** The effect of tannic acid on the angiogenic consequences of CXCL12/CXCR4 interaction was studied using an in vitro assay of capillary tube outgrowth. Endothelial cells, including BAECs, have been shown to express functional CXCR4 (29, 30), which was up-regulated by the angiogenic factors vascular endothelial growth factor, and bFGF (30, 31). In our experimental system, CXCL12, bFGF, or ECGS stimulation resulted in BAEC tube formation. Treatment with 0.5 μg/ml of tannic acid completely inhibited tube formation induced by CXCL12, whereas this concentration of tannic acid did not suppress tube formation induced by bFGF and ECGS (Fig. 9). Inhibition of bFGF and ECGS-induced BAEC tube formation could be observed when tannic acid concentration reached 10 μg/ml.
fractionation result suggested that the tannin contained in Lianqiao may be one of the components responsible for this activity.

The use of tannic acid-rich plants in folk medicine has a long history. The unconscious use of tannic acid probably dates back to the Neanderthals who treated burns with extracts of plants (42). Chinese medicine has continued and perfected this practice through the ages (43). Tannic acids have been shown to be responsible for some of the therapeutic efficacy of herbal medicines. For example, sanguiin H-11, a tannic acid purified from the Sanguisorba officinalis (Chinese herbal medicine Di Yu), is reported to be a potent inhibitor of rat neutrophil chemotactic response to CINC-1, platelet activating factor, and fMLP (44).

Our experiments showed that both aqueous and organic extracts of Lianqiao inhibited CXCL12 binding to CXCR4, thereby blocking CXCL12-induced chemotaxis. Purification by sequential chromatography of inhibitors of CXCL12/CXCR4 suggested that tannic acids are one of the active components in Lianqiao. Evaluation of the activities of commercially available tannic acid supported and verified this idea. Among an array of tested chemokines and chemoattractants (CXCL12, CCL2, CCL3, CCL5, C5a, and fMLP), tannic acid (10 μg/ml) selectively inhibited CXCL12-induced human monocyte chemotaxis. Tannic acid also blocked radiolabeled CXCL12 binding to THP-1 cells and human primary leukocytes (lymphocytes and monocytes). The difference in IC50 values observed between the binding assay and chemotactic assay may be attributable in part to the concentrations of CXCL12 used in these assays. In chemotactic assays, the concentration of CXCL12 was 100 ng/ml, which is substantial higher than the binding assay (0.96 ng/ml). Besides, when tannic acid was loaded only in the upper wells of chemotactic chamber with cells, it could be diluted by the medium in the lower wells, therefore requiring a relatively higher concentration of tannic acid to reach the inhibitory effect. The results could not be attributed to cytotoxic effects or non-specific conjugation of tannic acid on the proteins, because tannic acid did not show any effect on cell viability at concentrations that selectively inhibited chemotaxis and binding. Furthermore, the inhibitory effect of tannic acid on CXCL12 binding to THP-1 cells could be reversed by thoroughly washing tannic acid out of the cell suspension, also excluding the possibility of a toxic effect. Binding of a CXCR4 antibody to freshly isolated human monocytes was inhibited by treatment with tannic acid (whereas anti-CD14 binding was not inhibited). Our hypothesis is that tannic acid interferes with antibody binding to the receptor but does not down-regulate CXCR4 expression. This idea may be supported by the fact that washing out tannic acid from pretreated cells completely abrogates inhibition of 125I-labeled SDF-1α binding. Taken together, our observations favor the idea that tannic acid may interact with and change CXCR4 conformation and consequently interfere with CXCL12 binding to CXCR4.

Certain chemokines are involved in tumor growth and angiogenesis (45). Recently, CXCL12 and its receptor CXCR4 have been shown to play a pivotal role in breast tumor cells metastasis into the lung and bone marrow (5). Tannic acid also inhibited MDA231 cell migration induced by CXCL12, whereas the migration induced by EGF was not influenced by tannic acid, suggesting that tannic acid could block CXCL12-induced

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**DISCUSSION**

Chinese medicinal herb Lianqiao is the dried unripe fruit of Forsythia suspensa (Thunb.) Vahl (32), which is used primarily for treatment of tonsillitis, tonsillar pain, pharyngitis, and as an antipyretic and anti-inflammatory drug (33). Extracts of Lianqiao have been shown previously to have several activities including antibacterial (32, 34, 35), anti-inflammatory (36–38), blocking platelet activating factor binding to platelets (39), inhibiting nitric oxide production (40), and suppressing substance P-induced itch-scratch response (41). Our data showed that both aqueous and organic extracts of Lianqiao inhibited CXCL12 binding to the receptor and induced chemotaxis, whereas extracts of two other component herbal extracts in SHHL did not significantly inhibit CXCL12 function. The inhibitory activity of Lianqiao, therefore, may be an underlying mechanism for its anti-inflammatory efficacy. Our systemic
MDA 231 cell metastasis selectively. Although the proliferation of BAECs was inhibited by 10 µg/ml of tannic acid, capillary tube formation by BAECs induced by CXCL12 was significantly inhibited by 0.5 µg/ml of tannic acid. The same concentration of tannic acid did not affect basic fibroblast growth factor- or ECGS- induced tube formation, suggesting that tannic acid selectively antagonized the angiogenic activity of CXCL12.

Taken together, these data suggest that tannic acid is a CXCL12/CXCR4 inhibitor, and this activity may contribute to its anti-inflammatory and antitumor properties. Further elucidation of structure-activity relationship may be helpful for new drug design and development based on this activity. Because tannic acid is abundant in the plant kingdom and is present in the daily diet and because of the crucial role of CXCL12/CXCR4 in numerous physiological as well as pathogenic processes, inhibition of CXCL12/CXCR4 by tannic acid may exert profound influences on human health.

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